

**Original Research Article****Study of Efficacy of Manual Liquid Based Cytology (MLBC) in Fine Needle Aspiration Samples****Rahul Karode<sup>1</sup>, Anjali Singh<sup>2</sup>, K.K. Sharma<sup>3</sup>, Sanjeev Narang<sup>4</sup>, S.K. Nema<sup>5</sup>**

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**Abstract**

**Background:** Liquid Based Cytology (LBC) is defined as a method of preparing cytological specimens for microscopic evaluation in which the patient's aspirated specimen is suspended in a liquid medium, which is used to produce a thin layer of cells. The objective of our study was to prove the efficacy of Manual Liquid Based Cytology (MLBC) over Conventional Smear Cytology (CS) in Fine Needle Aspiration (FNA) samples including body fluids.

**Methods:** In this comparative study 100 FNA samples from various anatomical sites were assessed by both MLBC and CS technique under the criteria of cellularity, background, cellular preservation, nuclear preservation. These criteria are evaluated by Kruskal-Wallis test and p-value <0.001 is considered as statistically significant.

**Result:** MLBC technique shows better results as compare to CS in terms of cellularity and cellular preservation (p-value < 0.001) whereas MLBC doesn't show statistically significant difference in background (p-value = 0.412) and in nuclear preservation (p-value = 0.567).

**Conclusion:** This study though shows that MLBC is safe and less time-consuming technique, however it doesn't offer any diagnostic superiority over CS in the evaluation of FNA samples. We recommend CS as a gold standard technique with MLBC used as a supportive procedure in some cases.

**Keywords:** Manual Liquid-based Cytology; Conventional Cytology; FNA Samples and Body Fluids.

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**Introduction**

Liquid Based Cytology (LBC) is defined as a method for preparing cytological specimens for microscopic evaluation in which the patient's specimen is suspended in a liquid, which is used to produce a thin layer of cells. LBC leads to fewer false negative specimens, standardization of the preparation, better cell preservation, fewer inadequate samples, prevention of overly thick cell clusters, elimination of air drying artifact, removal of debris and

inflammation and allows Human Papilloma Virus (HPV) testing on remaining specimen. LBC has been largely employed in the evaluation of cervicovaginal specimens, replacing, in many countries, the Papanicolaou screening based on conventional smears (CS). Alleged advantages of LBC for gynecological cytology include improvement in specimen quality and adequacy, lower unsatisfactory rates, increased detection of precursor lesions, and the usage of residual samples for ancillary tests [1,2]. In general, studies that reported the use of LBC for body

fluids and Fine Needle Aspiration (FNA) specimens, including breast aspirates, found better cellular preservation, less cell overlapping and elimination of obscuring elements (blood, inflammatory cells, and cellular debris) in comparison to CS [3,4,5].

On the other hand, alterations in architecture and cellular morphology, as well as loss of informative background (stromal cells and extracellular material), have been described in FNA specimens prepared by LBC [3, 6, 7].

LBC in FNA samples can be performed on aspirates from different organs like salivary gland, thyroid, lymph nodes, breast, bone and soft tissue and other usual and guided FNAC samples. In a comparison study, usually, two slides will be made from the single subject. The first one is performed by the conventional preparation and the second one is by LBC preparation. Familiarity with artifacts is essential to avoid misinterpretations [8,9].

The aim of present study is to prove the efficacy of MLBC over Conventional Smear Cytology in Fine Needle Aspiration Samples including body fluids with additional objectives of to establish a suitable method for preparation of smears for cytological evaluation of aspirated materials and to establish the efficacy of MLBC over Conventional Cytological Examination.

## Materials & Methods

The present study is a comparative study, undertaken to study the efficacy of Manual Liquid Based Cytology over Conventional cytology in various Fine Needle Aspiration Samples. Cases from January 2016 to June 2017 constituted the subject material for the present study and total 100

cases with different fine needle aspiration samples from patients who attended the Out Patient as well as In-Patient Department of various clinical branches for FNAC investigation in Index Hospital attached to Index Medical College, Hospital & Research Center (IMCHRC), Khudel, in Indore were included.

For Fine Needle Aspiration Cytology (FNAC), two passes were performed according to standard procedure. The first pass was for CS and the second pass for the MLBC preparation. For CS slides were directly sent for staining after FNAC whereas for MLBC additional steps were followed before staining. After aspiration, the material was transferred in an alcohol-based preservative vial and kept for 30 minutes under refrigeration. The preservative vial was then placed in the centrifuge and centrifuged for 5mins @ 1500 – 2000 rpm. After centrifugation, from the pellet slides, were prepared by adding fixative (used for homogenous and even spread of cells on the slide). The smear was allowed to dry and then sent for staining. Leishman-Giemsa Stain and Papanicolaou (Pap) stains were used as staining method.

Both Conventional (CS) and MLBC smears were then studied and compared on the basis of 4 cytological criteria namely: Cellularity, Background, Cellular Preservation and Nuclear Preservation. The comparison was based on semi-quantitative scoring system (as described in Table 1). After scoring, Kruskal Wallis test was applied for obtaining p-value (p-value <0.001 is considered significant).

## Results

The Semiquantitative scoring system used in various FNA smears is shown in Table 1.

**Table 1:** Semiquantitative scoring system used in various FNA smears

Cytological Features	Score 0	Score 1	Score 2	Score 3
Cellularity	Absent	Mildly cellular	Moderately cellular	Markedly cellular
Background	Blood and debris present	Mildly Clear	Moderately Clear	Markedly Clear
Cellular Preservation	Poor	Fair	Good	Excellent
Nuclear Preservation	Poor	Fair	Good	Excellent

**Table 2:**

Site	Cytological Diagnosis	No. of Cases	CS		MLBC		Excluded Cases
			Benign	Malignant	Benign	Malignant	
Lymph Node		21					1 in LBC
	Reactive Lymphadenitis	9	9	0	9	0	
	Granulomatous Lymphadenitis	10	10	0	10	0	
Breast	Malignant	2	0	2	0	2	
		12					1 in LBC
	Fibroadenoma	3	3	00	3	00	
	Breast Abscess	3	3	00	3	00	
	Cystic Lesion	1	1	00	00	00	
IDC(Malignant)	5	0	5	0	5		

Thyroid		31						1 in LBC
	Nodular Goiter	17	17	00	16	00		
	Thyroiditis	5	5	00	5	00		
	Benign Cystic Lesion	4	4	00	3	00		
	Follicular Neoplasm	2	0	2	0	2		
	Papillary Neoplasm	2	0	2	0	2		
	Huthle Cell Adenoma	1	0	1	0	1		
Salivary Gland		4						1 in LBC
	Pleomorphic Adenoma	2	2	0	2	0		
	Parotitis	1	1	0	1	0		
	Mucoepidermoid Ca	1	0	1	0	0		
Other S.T. Swellings		8						1 in LBC
	Abscess	5	5	0	5	0		
	Epidermal Cyst	1	1	0	1	0		
	Lipoma	1	1	0	0	0		
	Sq. Cell Carcinoma	1	0	1	0	1		
Body Fluids		24						1 in LBC
	Transudate	9	9	0	9	0		
	Exudate	12	12	0	12	0		
	Malignant	3	9	3	0	3		

**Table 3:**

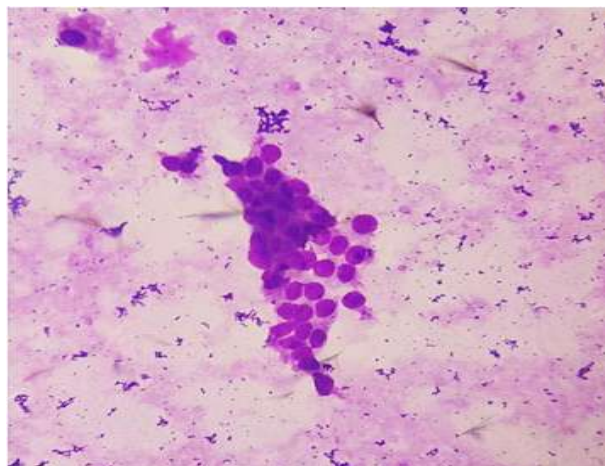
FNAC Features	Present Study.		P. Arul. (2015)		Mygdakos et al. (2009)		Koybasioglu et al. (2008)	
	Kruskall Wallis Rank Test		Willcoxon Signed Rank Test		Willcoxon Signed Rank Test		ANOVA	
	Z	P	Z	P	Z	P	F	P
Cellularity	-5.98	<0.001	-1.414	0.157	-1.352	0.131	5.250	0.023
Background	0.82	0.412	-1.732	0.083	-1.997	0.057	77.712	0.000
Cellular	-6.15	<0.001	-3.234	0.001	-3.197	<0.001	3.869	0.051
Preservation/ Cytoplasmic details								
Nuclear	0.57	0.567	-3.494	<0.001	-3.197	<0.001	0.720	0.398
Preservation/ Nuclear Details								

A total of 100 cases were studied which were distributed as, 21 cases of lymph node, 12 cases of breast, 31 cases of thyroid, 4 cases of the salivary gland, 8 cases of other soft tissue swelling and 24 cases of body fluids shown in Table 2.

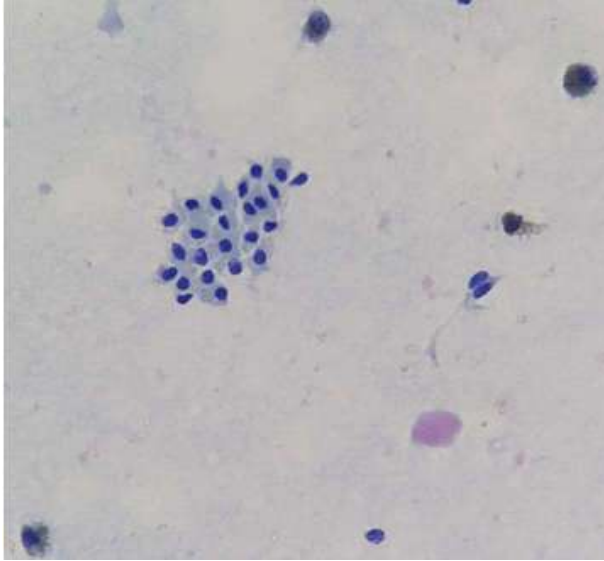
According to the Kruskal-Wallis test, the present study showed that MLBC preparations were superior to CS preparations in view of cellularity ( $p < 0.001$ ), and cellular

preservation ( $p < 0.001$ ). However, no statistically significant differences were found between LBC and CS preparations with regard to the informative background ( $p = 0.412$ ), and nuclear preservation ( $p = 0.567$ ).

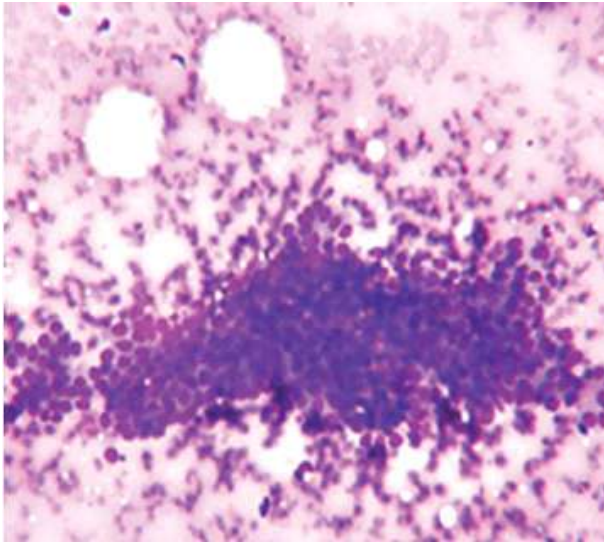
Table 3 shows Comparison of MLBC and CS preparations of the present study and the published studies.



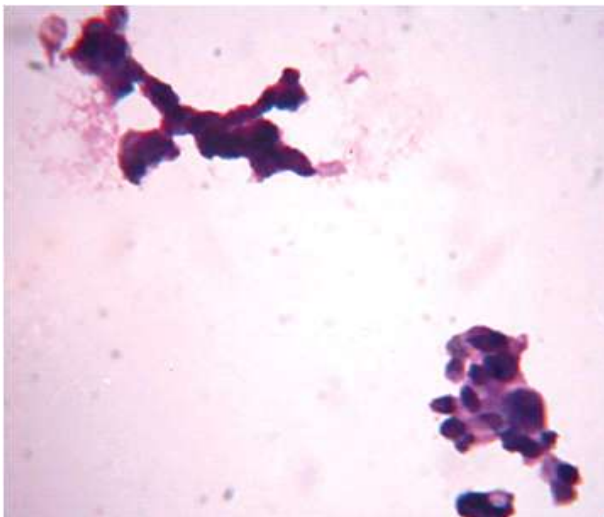
**Fig. 1:** Thyroid FNAC (CS) 10x



**Fig. 2:** Thyroid FNAC (MLBC) 10x



**Fig. 3:** Breast FNAC (CS) 10x



**Fig. 4:** Breast FNAC (MLBC) 10x

## Discussion

Liquid-based cytology (LBC) was first approved by FDA in 1996 [10] and initially it was for evaluating gynecological samples only. But later as the procedure evolved it has been found that LBC also holds good for Non-gynecological specimens also like FNAC and Body fluids and various studies had been carried out by different authors for the Non-gynecological specimen with variable results and conclusions are postulated by them.

As we know there are various problems and drawbacks encountered in case of conventional FNAC and Body fluids smears. Many of these conventional smears show cells in as singly scattered and in clusters as well as groups but mostly they are admixed with confounding factors like blood, debris, exudates and necrosis which hinders the information pathologist want to conclude for diagnosis and hence leads to inadequate and unsatisfactory smears. These disadvantages of conventional cytology are encountered by pathologists from last many years. To counter these drawbacks Liquid Based Cytology technique has been evolved which preserves the cells in liquid medium and removes most of the debris, blood, exudates and necrotic material either by the principle of filtration or density gradient interpretation.

Hence, from the view of pathologists, the advantages of LBC are minimal confounding factors, excellent cellular preservation, decreasing artifacts and evenly spread monolayered sheets of cells.

These features are more obvious in automated LBC whereas they are subtle in cases of manual LBC.

In our present study, we had taken in consideration of FNAC from various anatomical sites namely, Lymph node, Thyroid, Breast, Salivary glands, other soft tissue swellings as well as body fluids.

In cases of lymph node, FNAC presence of lymphoglandular bodies in the background is the most important identification feature which with the use of LBC technique in the present study are evident only in half of the cases and it was difficult to make out these bodies in other cases.

Also, LBC technique doesn't prove superior to CS in the nuclear preservation and hence it is still difficult to make the diagnosis of malignant cases. However, in the present study, LBC preparation shows some advantages also like an easy visualization of lymphoid cells and presence of these cells in monolayered sheets. Garbar et al. [11] had performed a study on FNAC of lymph node with both the procedures and they conclude that despite the cost the interpretation of lymph node FNAC are almost same between LBC and CS but according to our study to form a more definitive diagnosis it is better to use both CS and LBC.

In cases of thyroid FNAC, to distinguish between malignant and benign diseases identification of colloid is very important. In CS the colloid is present as round, dense clumps or as globular masses which is very evident but in LBC amount of colloid decreases may be due to suspending the aspirate in liquid media and it appears fragmented. But the advantages of LBC technique in thyroid FNAC is presence of follicular cells in small monolayered groups with better cellular preservation, hyperplastic and hurthle cell changes as compared to CS. The background is slightly better in LBC as compared to CS and some of the cases show clearer background devoid of hemorrhagic material. The major disadvantage of LBC which we found in the present study is that the nuclear features are not so striking and therefore in cases of papillary neoplasm where nuclear features are of utmost importance for diagnosis we have to interpret things more attentively and cautiously. Nasuti JF, Tam D, Gupta PK et al. [12] concluded in their study that LBC in thyroid FNAC specimens were more often less than optimal. In the present study it is easier to form diagnosis in benign cases with slight experience and expertise but for malignant cases, it is better to use CS and LBC should be used as a supportive technique and cost effectiveness should also be taken in account of.

As far as breast FNAC specimens are concerned cases of breast carcinoma show superior cellularity and cellular preservation with LBC whereas the background is comparable with CS and nuclear preservation is inferior to CS. Hence we can interpret that both the techniques show almost comparable results. In fibroadenoma, stromal cells are either lost or decreased substantially but ductal cells aggregates are visualized better in LBC as compared to CS. Diagnosis of fibroadenoma depends on the presence of both the cellular entities, therefore, it is better to make the diagnosis on CS rather than LBC. In cases of breast abscess LBC show clearer background which is devoid of necrotic debris and blood hence there is the better visualization of inflammatory cells along with the even spread of ductal epithelial cells in small groups or singly scattered. Bedard YC et al. [7], performed study in which he compares the results of Breast FNAC specimens with both LBC and CS methods and he concludes that there is no significant difference in diagnostic accuracy and in present study their results are comparable with malignant cases but we observe low diagnostic rate in fibroadenoma and higher in cases of breast abscess. Similar results are concluded by Ryu H.S., Park I.A. et al. [5] where they stated that LBC technique is reliable for evaluation of breast lesions but its diagnostic accuracy is equivalent to CS.

When we evaluate salivary gland lesions in the present study, in cases of Pleomorphic Adenoma we found cellularity is higher in CS as compare to LBC along with

better preservation of stromal fragments and chondromyxoid matrix. There is a better nuclear preservation of ductal and myoepithelial cells in CS as compare to LBC. Also in mucoepidermoid carcinoma which is cystic neoplasm LBC show very sparse cellularity which is unsatisfactory for evaluation hence diagnosis was made by the help of CS. These results are comparable to Parfitt et al. [13] and Tripathy K. et al. [14].

As we assess the other soft tissue swellings in the present study, the cases of abscess show the diagnostic accuracy of LBC comparable to CS with advantages of the clearer background by removing obscuring confounding factors and better cellular preservation. In cases of an epidermal cyst and squamous cell carcinoma where cellular yield is low CS show superior features than LBC.

We also consider the assessment of body fluids aspirated from various sites in our study viz. pleural fluid, ascitic fluid and synovial fluid. In benign cases, LBC shows better cellular preservation and clear background in the majority of cases whereas nuclear preservation is better in CS and cellularity is more or less similar in both the techniques. In cases where cellular yield is low LBC comes inferior to CS. During the analysis of malignant cases, CS is superior to LBC in terms of nuclear preservation and cellularity except for the background. Nasuti et al. [12] in their study concluded that body cavity fluids show generally satisfactory results with LBC but according to our study satisfactory results are present in benign cases but for malignant cases, it is better to use CS with LBC as supportive technique.

## Conclusion

MLBC technique is easy and superior to CS in terms of cellularity and cellular preservation but it requires more experience and knowledge of artifacts during the interpretation and overall experience with this study was that diagnostic yield increases in MLBC with body fluids. Finally, we can conclude that despite the advantages offered by MLBC its efficacy is inferior to CS and it is better to use CS as gold standard whereas MLBC is used in concordance with CS according to the cases to achieve optimal diagnostic yield.

*Finding:* None

*Competing Interests:* None

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